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Renal tubular function in glycerol-induced acute renal failure

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Renal tubular function in glycerol-induced acute renal failure. The purpose of this study was to examine proximal and distal tubular function in rats with nonoliguric, myohemoglobinuric acute renal failure (ARF). ARF was induced with glycerol (50%, 10 ml/kg of body wt, i.m.), and renal function was studied 24 hours after glycerol or saline (controls) injection. Glycerol injection caused a 50 to 90% depression in GFR and a significant rise in blood urea nitrogen concentration. Animals with ARF exhibited glycosuria with normal blood-sugar levels and a striking depression in tubular glucose reabsorption per milliliter of GFR. The capacity to reabsorb bicarbonate (mEq/liter GFR) was intact at normal blood bicarbonate levels, but was markedly depressed when blood bicarbonate was raised. The tubular maximum for para-aminohippurate (PAH) secretion and the renal extraction fraction of PAH were strikingly depressed in rats with ARF. Distal acidification as assessed by the urine-to-blood gradient of P_{CO_2} (U-B P_{CO_2}) was normal both during maximal alkalinization of the urine with bicarbonate (urine pH, approximately 7.8) or during neutral phosphate infusion (urine pH, approximately 7.0). Net acid excretion per milliliter GFR and minimal urine pH (< 5.5) following 3 days of ammonium chloride ingestion was similar in control and ARF animals. Potassium excretion was intact in ARF animals, and hyperkalemia did not occur. Minimal and maximal urinary osmolality were significantly altered in animals with ARF. Cortical and outer medullary Na-K-ATPase specific activities were significantly depressed in ARF rats. This occurred as a consequence of enzyme loss and not secondary to alterations in enzyme kinetics or absolute tubular sodium reabsorption. Light and electron microscopy showed diffuse proximal tubular damage, whereas glomeruli and distal tubules were intact. These data demonstrate that glycerol injection produces a diffuse proximal tubular transport defect associated with histologic and enzymatic alterations.

Fonction tubulaire rénale au cours de l'insuffisance rénale aiguë déterminée par le glycérol. Le but de ce travail a été l'étude de la fonction tubulaire proximale et distale chez des rats atteints d'insuffisance rénale aiguë (ARF) myoglobinurique, non oligurique. L'ARF a été déterminée par le glycérol (50%, 10 ml/kg poids corporel, i.m.) et la fonction rénale a été étudiée 24 heures après la perfusion de glycérol ou de soluté. L'injection de glycérol a déterminé une diminution de 50 à 90% du débit de GFR et une augmentation significative de la concentration plasmatique d'azote uréique. Les animaux atteints d'ARF ont une glycosurie avec une glycémie normale et une dépression importante de la réabsorption tubulaire du glucose par millilitre de GFR. La capacité à réabsorber bicarbonate (mEq/litre GFR) est intacte aux concentrations normales de bicarbonate mais fortement abaissée quand la concentration de bicarbonate plasmatique est augmentée. La sécrétion tubulaire maximale de para-aminohippurate (PAH) et l'extraction rénale du PAH sont diminuées chez les rats en ARF. L'acidification distale évaluée par le gradient urine/sang de P_{CO_2} (U-B P_{CO_2}) est normale aussi bien au cours de l'alcalinisation maximale de l'urine par bicarbonate (pH de l'urine, approximativement 7,8) qu'au cours de la perfusion de phosphate neutre (pH de l'urine, approximativement 7,0). L'excrétion nette d'acide rapportée au ml de GFR et le pH minimal de l'urine (< 5,5) après trois jours d'ingestion de chlorure d'ammonium sont semblables chez les contrôles et les animaux en ARF. L'excrétion de potassium est conservée chez les animaux en ARF et il ne survient pas d'hyperkaliémie. Les osmolalités urinaires minimale et maximale sont significativement modifiées chez les animaux en ARF. Les activités spécifiques de la Na-K-ATPase corticale et médullaire sont significativement abaissées chez les rats en ARF. Ceci est la conséquence d'une perte d'enzyme et non de modifications de la cinétique enzymatique ou de la réabsorption absolue de sodium. La microscopie photonique et électronique montre des lésions tubulaires proximales diffuses alors que les glomérules et les tubes distaux sont indemnes. Ces résultats démontrent que l'ingestion de glycérol produit une perturbation globale du transport proximal associée á des modifications histologiques et enzymatiques.

The study of experimental and human acute renal failure (ARF) has to date largely concentrated on pathogenic and pathophysiologic aspects of filtration failure [1, 2]. The acute fall in GFR in this syndrome is accompanied, however, by a variety of defects of tubular function. These functional derangements have been only sporadically examined. In this study, we examined proximal and distal tubular function using conventional clearance techniques in the glycerol model of ARF in rats. The functional changes observed were correlated with biochemical and histologic changes.

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Methods

Male Sprague-Dawley rats (R. Locke, Chicago, Illinois), each weighing between 250 and 450 g, were used in all experiments. The animals ate regular rat chow and had free access to tap water. ARF was induced by injecting 10 ml/kg of glycerol (50% in tap water) into the muscles of both hindlegs [3]. Control animals were injected with an equal volume of normal saline (10 ml/kg, i.m.). All studies of renal function were carried out 24 hours after the injection of glycerol or saline. At the conclusion of each clearance study, kidneys were rapidly removed, drained of blood, and processed for enzyme assays and histologic examination (see below). For the renal function studies, the animals were anesthetized with sodium pentobarbital, 30 mg/kg, i.p. (Nembutal, Abbott Laboratories, North Chicago, Illinois) and placed on a heated board. Their rectal temperatures were maintained at 37 to 38° C. A tracheostomy was performed, and two jugular veins and one carotid artery and the bladder were cannulated with PE-50 tubing.

The GFR was calculated from the clearance of ^{125}I-iothalamate (Glofil, Abbott Laboratories, North Chicago, Illinois) as previously reported [4]. Normal saline containing ^{125}I-iothalamate (0.75 μCi/ml) was infused with a constant infusion pump (model 355, Sage Instruments, Cambridge, Massachusetts) at a rate of 20 μl/min. After an equilibration period of 60 min and i.v. replacement of intraoperative fluid losses by Ringer's solution (equivalent to 1% body weight), urine was collected under mineral oil during two to three control (for definition see below) and two to three experimental periods of 30 to 60 min's duration. Blood from the carotid artery was obtained at the midpoint of each clearance period. Blood and urinary losses were replaced by i.v. Ringer's solution. Urine volume was determined gravimetrically after correction for specific gravity. ^{125}I-iothalamate-containing samples were counted in a Beckman gamma counter (Biogamma, Beckman Instruments, Irvine, California). Electrolytes, blood urea nitrogen (BUN), glucose, phosphate, pH, P_{CO_2}, osmolalities, and para-aminohippurate (PAH) were determined in blood and urine as previously reported [5, 6].

Glucose titration. In 10 rats with ARF and in 8 control rats, 50% glucose in water was infused following two to three control periods. The glucose infusion was increased in a stepwise fashion, allowing stabilization of plasma glucose at increasing concentrations. At each concentration of plasma glucose, one or two clearance samples were collected.

Bicarbonate titration. In 8 experimental rats, (that is, 24 hours after glycerol) and 6 control rats, 0.9 M sodium bicarbonate was infused i.v. following collection of two to three control samples. The sodium bicarbonate infusion was increased in a stepwise fashion, allowing stabilization of blood bicarbonate at increasing levels. At each concentration of blood bicarbonate, one or two clearance samples were collected. A maximally alkaline urine (urine pH, close to 7.8) was achieved by raising blood bicarbonate to approximately 40 mEq/liter.

PAH secretion. In 12 ARF and 11 control rats, sodium PAH (Merck, Sharp and Dohme, Westpoint, Pennsylvania), 4 mg/ml in normal saline, was infused at a rate calculated to maintain the plasma concentration around 3 mg/dl. Thereafter, the plasma concentration of PAH was increased in a stepwise fashion, by using an infusate containing PAH at a concentration of 12 mg/dl. At each level of PAH infusion (after 30 min of equilibration), one or two sample collections were obtained. At the conclusion of each study (5 control and 5 ARF animals), a small sample of blood from either renal vein (27-gauge needle) was collected so as to allow assessment of the PAH extraction fraction. For all PAH calculations, it was assumed that 20% of the plasma PAH was protein bound, and appropriate corrections of plasma PAH concentrations were made accordingly [7].

Phosphate infusion in animals with moderately alkaline urine. After one to two control periods, 6 ARF and 6 control rats were infused with 0.3 M neutral phosphate (dibasic-to-monobasic sodium phosphate molar ratio of 4:1; pH, 7.4) at an increasing rate of 1 to 5 ml/hr. After 30 min of equilibration, one to two clearance collections were obtained at each rate of phosphate infusion. Sodium bicarbonate (0.9 M) was simultaneously infused to achieve a stable urine pH between 6.8 and 7.4, and P_{CO_2} in blood and urine was measured throughout the experiment.

Net acid excretion. Twelve rats were given 1.5% ammonium chloride as drinking fluid for 5 days preceding the study. Twenty-four hours prior to study, 5 rats were injected with glycerol (ARF), and 7 rats were injected with 0.9% saline (control). The animals were prepared for clearance study as described above, and three to four clearance collections were obtained. The titratable acidity was assessed by the amount of 0.1 N sodium hydroxide used to titrate 1 ml of urine from urine with a pH of

up to 7.4. Ammonia was measured by the formaline titrimetric method of Cunarro and Weiner [8]. Net acid excretion was calculated as the sum of titratable acidity and ammonium excretion minus the urinary bicarbonate excretion.

Free water clearance (C_{H_2O}). In 8 rats with ARF and 6 controls, C_{H_2O} and minimal urinary osmolality (U_{min}) was determined. For this purpose, all rats were only lightly anesthetized with ketamine hydrochloride (Ketalar, Parke-Davis, Detroit, Michigan), 1 mg/kg of body wt, i.p. After induction of anesthesia, a pediatric feeding tube was perorally introduced into the stomach, and a 0.225% saline and 1.5% glucose solution was slowly instilled (equivalent to 8% body wt). In addition, a solution of 0.225% sodium chloride was infused i.v. at a rate equal to the urine flow. After achieving maximal depression in urine osmolality (U_{min}) and after 60 min of equilibration, several blood and urine samples were collected while a steady-state water diuresis was maintained. C_{H_2O} was calculated as $V-C_{Osm}$, where V is the urine flow and C_{Osm} is the osmolar clearance, both expressed in milliliters per minute. [7].

Free water reabsorption ($T^c_{H_2O}$). In 10 rats with ARF and 6 controls, $T^c_{H_2O}$ and maximal urinary osmolality (U_{max}) was determined. The animals were water deprived for 18 hours prior to study and received 1 U, s.c., of Pitressin tannate in oil® (Parke-Davis, Detroit, Michigan), at 12 and 2 hours before the first urine was collected. The osmolality of the first spontaneously voided urine (morning sample) was taken as U_{max}. Thereafter, the animals were anesthetized and cannulated as described above. All rats were infused with 3% sodium chloride at progressively increasing rates, from 0.1 to 1 ml/min, in a period of approximately 2 to 3 hours to raise osmolar clearance. Aqueous Pitressin was infused continuously at a rate of 20 mU/hr. Four to six clearance collections were obtained. $T^c_{H_2O}$ was calculated as $C_{Osm}-V$ [7].

Aortic constriction. Seven normal rats had a silver clip applied to the abdominal aorta above the renal arteries to reduce the aortic diameter by 60%. Six normal rats were sham operated, and 7 remained unoperated. The data from the intact and sham-operated rats were combined and reported as such, for they were not statistically different. An additional 21 animals were injected with glycerol as detailed above. GFR, electrolyte excretion, and renal Na-K-ATPase activity (see below) was measured in all these animals 24 hours after surgery or glycerol injection.

Histology. The kidneys of 6 randomly chosen ARF (from bicarbonate titration and glucose titration groups) and 6 control animals were fixed by in vivo perfusion with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH, 7.4) as previously described [9].

After completion of perfusion fixation, tissue slices for light microscopy were transferred into 10% fresh formaldehyde and processed for sectioning and staining according to routine methodology. Tissue slices corresponding to those examined by light microscopy remained for an additional 2 hours in the glutaraldehyde-phosphate solution. Thereafter, the tissue was postfixed in 2.0% osmium tetroxide in 0.1 M phosphate buffer (pH, 7.4) and dehydrated in a series of acetone solutions. The tissue was then infiltrated with a 1:1 solution of Epon 812®/100% acetone, pure Epon 812, and finally embedded and polymerized in Epon 812. Tissue was thin-sectioned at approximately 60 nm; the sections were placed on 200-μ mesh copper grids and stained with uranyl acetate and lead citrate. The tissues were examined on an RCA EMU 4 electron microscope.

Enzyme studies. Renal Na-K-ATPase activity was determined in 13 control animals (6 sham operated for aortic constriction and 7 left intact), in 7 animals with aortic constriction (see above: *Aortic constriction*) and in 21 animals with ARF. All kidneys were rapidly removed at the end of each clearance study (25 to 27 hours after surgery or glycerol injection). The details of tissue dissection, preparation of whole homogenates from cortex, outer medulla and papilla, and Na-K-ATPase assay have been previously reported [4]. The Na-K-ATPase specific-activity was expressed in micromoles of inorganic phosphate per milligram of protein per hour. Inorganic phosphate was determined by the method of Fiske and SubbaRow [10], and the protein content of tissue suspensions by the method of Lowry et al [11]. The linearity of ATPase activity (total and ouabain-sensitive) with time or enzyme concentration was assessed by varying either the incubation time from 1 to 20 min or the volume of tissue homogenate (cortex and outer medulla from control, ARF, and aorta-constricted animals) from 0.05 to 0.3 ml/incubation, respectively. The correlation coefficient (r) for both maneuvers was at least 0.99, indicating a high degree of linearity of the enzyme assay when performed under the described conditions. Because animals with ARF (see below in Results section) showed a marked depression in Na-K-ATPase specific activity, both in cortex and

Table 1. Glucose reabsorption in control animals (C) and animals with acute renal failure (ARF)[a]

Experimental period	GFR *ml/min*		$P_{glucose}$ *mg/dl*		T_G/GFR *mg/ml*		$U_{glucose}$ *mg/dl*		FE_{Na} *%*		BUN *mg/dl*	
	C	ARF	C	ARF	C	ARF	C	ARF	C	ARF	C	ARF
Control	1.9 ±0.2	0.4[e] ±0.1	133 ±5	164 ±17	1.33 ±0.05	0.81[c] ±0.18	45 ±12	1,648[d] ±368	0.3 ±0.1	2.9[d] ±0.7	13.2 ±1.5	39.0[e] ±3.4
P	NS	NS	<0.001	<0.001	<0.001	<0.01	<0.001	<0.001	NS	NS		
Glucose loading	1.9 ±0.3	0.4[e] ±0.1	538 ±64	546 ±43	3.50 ±0.27	1.52[e] ±0.24	6,600 ±1,050	3,943[b] ±500	0.7 ±0.4	6.3[b] ±2.4	—	—

[a] Values are the means ± SEM (N = 8 control animals, 10 ARF animals). Abbreviations used are $P_{glucose}$, plasma glucose concentration; T_G/GFR, tubular glucose reabsorption per ml GFR; $U_{Glucose}$, urinary glucose concentration; FE_{Na}, fractional sodium excretion; BUN, blood urea nitrogen.

[b] $P < 0.05$, compared with control.

[c] $P < 0.02$, compared with control.

[d] $P < 0.01$, compared with control.

[e] $P < 0.001$, compared with control.

outer medulla, these tissues were subjected to kinetic analysis. The apparent Michaelis-Menten constants (K_m) for sodium, potassium, and ATP were determined from cortical and outer medullary homogenates from control, ARF, and aorta-constricted rats. The sodium dependence of the Na-K-ATPase activity was determined by varying the sodium concentration in the reaction mixture between 0 and 150 mM, with Tris-ATP-magnesium as substrate. The potassium dependence of the Na-K-ATPase was assessed by varying the potassium concentration in the incubation medium between 0 and 20 mM. Total ATPase and magnesium-ATPase were measured as above. The initial velocity of the ATPase reaction as a function of ATP concentration was determined by varying the initial ATP concentration between 0 and 6 mM. Magnesium was added separately from ATP such that the final concentration in the reaction mixture was likewise 0 to 6 mM. Linear conversion according to Hofstee [12] of the sodium, potassium, and ATP data demonstrated that the apparent Michaelis-Menten constants for cortical and outer medullary enzyme for all three groups were statistically indistinguishable[1], and that the reduction in specific Na-K-ATPase activity in ARF resulted from differences in enzyme concentration, that is, V_{max}.

Reagents and calculations. Analytical grade reagents were used whenever possible. Ouabain and the sodium and Tris salts of ATP (vanadate-free) were purchased from Sigma Chemical Corp. (St. Louis, Missouri).

Results are presented as the means ± SEM. Enzymatic data were analyzed according to Hofstee [12] and Dixon and Webb [13]. Linearity was assessed by linear regression analysis, and statistical significance of differences between group means was determined by the Student's t test. P values less than 0.05 were considered significant.

Results

Glucose reabsorption. The results from this experiment are summarized in Table 1 and Fig. 1. The animals with ARF had a marked depression in GFR and a significant rise in BUN. Fractional sodium excretion (FE_{Na}) prior to glucose loading differed significantly, whereas blood glucose concentrations before and after glucose loading were comparable in ARF and control rats. Figure 1 clearly illustrates the striking depression in tubular reabsorption of glucose per milliliter of GFR (T_G/GFR) seen in ARF rats both at low and elevated concentrations of plasma glucose. This marked depression of the renal threshold for glucose was reflected by glycosuria at low plasma glucose concentrations. In contrast, control animals excreted only small quantities of glucose when the plasma glucose concentration was in the normal range.

Bicarbonate reabsorption. The data from this experiment are summarized in Table 2 and Fig. 2. The GFR in animals with ARF was markedly depressed, and their BUN was significantly elevated when compared with control animals. Fractional chloride excretion (FE_{Cl}) before bicarbonate loading was higher in ARF animals. Following bicarbonate loading, FE_{Cl} rose significantly in control and ARF animals. The blood bicarbonate concentration before

[1] The apparent Km for sodium in cortical tissue of ARF rats was 15 ± 0.8 mEq (NS, vs. 15.7 ± 0.6 for control and 15.4 ± 0.7 for coarctation group); Km for potassium in ARF cortex was 1.8 ± 0.2 mEq (NS, vs. 1.7 ± 0.1 for control and 1.7 ± 0.1 for coarctation group). The Km for ATP in ARF cortex was 0.3 ± 0.1 mM (NS, vs. 0.3 ± 0.1 for control and 0.3 ± 0.1 for coarctation group). Data from outer medulla were similar.

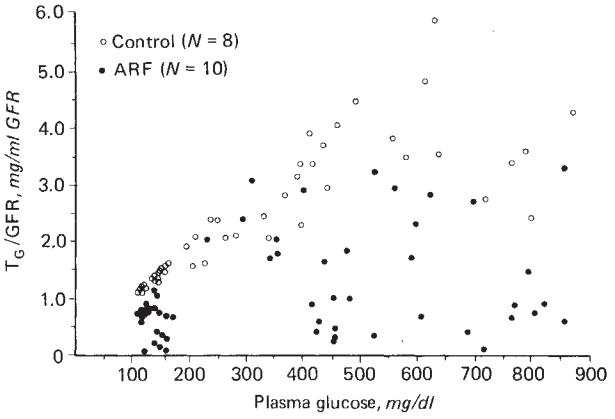

Fig. 1. *Plot of tubular glucose reabsorption per milliliter of GFR vs. plasma glucose concentration. Open circles* represent experimental points from 8 controls, and *closed circles* are those of 10 ARF animals.

bicarbonate loading and at high blood bicarbonate concentrations was comparable in both groups of animals. Figure 2 shows that ARF and control animals reabsorb bicarbonate completely up to a blood bicarbonate concentration of approximately 26 mEq/liter. The tubular capacity for bicarbonate reabsorption (T_{HCO_3}) above this point levels off in ARF animals, whereas control animals show a further almost linear increase in T_{HCO_3}/GFR as blood bicarbonate is raised. Thus, rats with ARF exhibited a marked reduction only in Tm HCO_3/GFR, for the renal threshold for HCO_3 was unaltered. The GFR in bicarbonate control animals was noted to be higher than that in glucose control rats. This resulted most probably from differences in body weight (bicarbonate controls weighed 350 ± 35 g, and glucose controls, 250 ± 30 g, $P < 0.05$).

PAH secretion. These experiments are summarized in Table 3 and Fig. 3. Both the GFR and BUN values of ARF animals differed significantly from those of controls. The tubular secretion of PAH at low and high concentrations of free PAH was markedly depressed in ARF animals. PAH secretion in ARF animals did not rise during PAH loading. In contrast, control animals exhibited a fivefold rise in PAH secretion with PAH loading. The clearance of PAH was low and fell with PAH loading in ARF animals. The PAH extraction fraction determined during PAH loading was only 10% in ARF animals as compared with 87% in control animals.

Urine-blood (U-B) P_{CO_2} during bicarbonate loading. The data from these experiments are summarized in Table 4 (also see Table 2). U-B P_{CO_2} before or after bicarbonate loading was similar in control and ARF animals. Urinary pH, bicarbonate, and phosphate concentrations were not different in ARF and control animals, both before and after bicar-

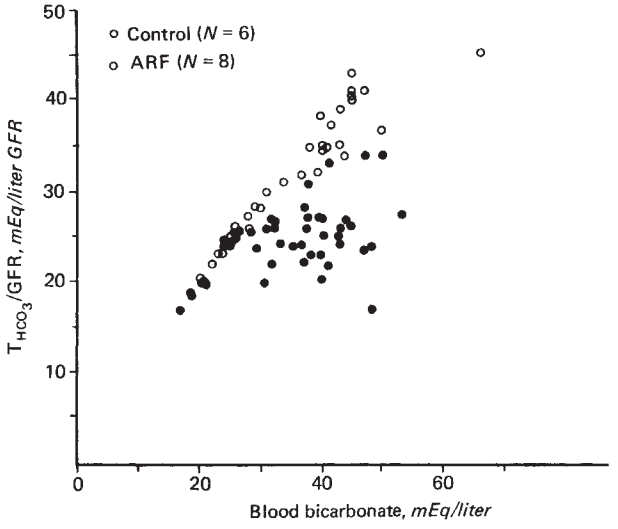

Fig. 2. *Plot of tubular bicarbonate reabsorption per liter of GFR vs. blood bicarbonate concentration.* For symbol identification, see legend to Fig. 1.

Table 2. Bicarbonate reabsorption in acute renal failure[a]

Experimental period	GFR *ml/min*		T_{HCO_3}/GFR *mEq/liter*		FE_{Cl} %		Blood HCO_3 *mEq/liter*		Blood P_{CO_2} *mm Hg*		BUN *mg/dl*	
	C	ARF	C	ARF	C	ARF	C	ARF	C	ARF	C	ARF
Control	3.0	1.1[c]	23.4	22.7	0.8	1.6[b]	24.0	23.0	39.0	41.8	17.4	38.3[c]
	±0.3	±0.3	±0.8	±1.2	±0.2	±0.4	±1.2	±1.2	±0.9	±3.8	±1.7	±5.6
P	NS	NS	<0.001	<0.05	<0.05	<0.01	<0.001	<0.001	NS	NS		
Bicarbonate loading	2.6	1.0[c]	36.3	25.7[d]	5.3	10.2[b]	43.3	39.7	38.7	41.6	—	—
	±0.2	±0.3	±1.9	±1.3	±1.9	±1.5	±1.2	±1.2	±1.1	±1.4		

[a] Values are the means ± SEM ($N = 6$ control animals, 8 ARF animals). Abbreviations not defined in Table 1 are: T_{HCO_3}/GFR, tubular bicarbonate reabsorption per liter GFR; FE_{Cl}, fractional chloride excretion.

[b] $P < 0.05$, compared with controls.

[c] $P < 0.01$, compared with controls.

[d] $P < 0.001$, compared with controls.

Table 3. PAH secretion in acute renal failure[a]

Experimental period	GFR *ml/min*		Free plasma PAH *mg/dl*		PAH secretion *mg/min*		C_{PAH} *ml/min*		EF_{PAH} *%*		BUN *mg/dl*	
	C	ARF	C	ARF	C	ARF	C	ARF	C	ARF	C	ARF
Low PAH	2.2	0.2[e]	2.0	5.3[e]	0.08	0.025[b]	7.8	0.71[e]	—	—	17.5	84.1[e]
	±0.3	±0.07	±0.3	±0.7	±0.02	±0.01	±1.4	±0.16			±1.0	±9.9
P	NS	NS	<0.001	<0.001	<0.001	NS	<0.02	<0.01				
High PAH	2.1	0.3[e]	35.1	71.9[e]	0.50	0.032[c]	4.81	0.33[c]	87.4	10.4[e]	—	—
	±0.2	±0.08	±4.9	±7.3	±0.04	±0.01	±0.58	±0.09	±2.3	±3.1		

[a] Values are means ± SEM (N = 11 control animals, 11 ARF animals). Abbreviations not defined in Table 1 are: C_{PAH}, PAH clearance; EF_{PAH}, PAH extraction fraction (data from 5 control and 5 ARF animals).
[b] $P < 0.05$, compared with controls.
[c] $P < 0.02$, compared with controls.
[d] $P < 0.01$, compared with controls.
[e] $P < 0.001$, compared with controls.

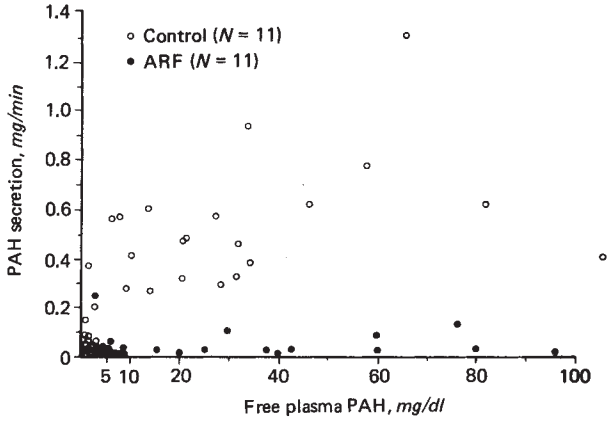

Fig. 3. *Plot of PAH secretion vs. the concentration of free plasma PAH.* For symbol identification, see legend to Fig. 1.

bonate loading. The fractional water excretion (V/GFR) was significantly higher in rats with ARF. These data indicate that urinary acidification as reflected by the urine-to-blood P_{CO_2} gradient remains intact in animals with ARF.

U-B P_{CO_2} during phosphate infusion. The data from this group are summarized in Table 5 and Fig. 4. The GFR was markedly depressed in animals with ARF. U-B P_{CO_2} during neutral phosphate infusion rose significantly in both control and ARF animals. Urinary pH and fractional sodium excretion were comparable in both groups. The urinary concentration of phosphate (U_{PO_4}) before phosphate infusion was significantly higher in ARF animals, whereas it rose in control animals to a higher final level when phosphate was infused. The high U_{PO_4} concentrations in ARF animals (before phosphate loading, control periods) was associated with a high fractional phosphate excretion and a significantly lower capacity to reabsorb phosphate per liter GFR (0.9 ± 0.3 in ARF, and 2.3 ± 0.1 mmoles/liter in controls, $P < 0.005$). The tubular reabsorption of phosphate (per liter GFR) was similar in control and ARF rats when phosphate was infused. The plasma phosphate concentration was not different in control and ARF animals. Figure 4 demonstrates that U-B P_{CO_2} rises linearly as the U_{PO_4} is raised both in ARF and control animals. Linear regression analysis and analysis of covariance of these data (for the range where U_{PO_4} in control and ARF animals overlaps) demonstrated that the line obtained in ARF

Table 4. Urine-blood P_{CO_2} in acute renal failure during sodium bicarbonate administration[a]

Experimental period	GFR *ml/min*		U-B P_{CO_2} *mm Hg*		U_{HCO_3} *mEq/liter*		Urine pH		U_{PO_4} *mmoles/liter*		V/GFR × 100 *%*	
	C	ARF	C	ARF	C	ARF	C	ARF	C	ARF	C	ARF
Control	3.0	1.1[c]	−3.8	−4.6	0.39	0.23	5.54	5.64	24.8	34.5	0.8	4.1[b]
	±0.3	±0.3	±1.1	±1.6	±0.02	±0.01	±0.22	±0.13	±11.6	±2.7	±0.2	±0.9
P	NS	NS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	NS	<0.001	<0.02	<0.01
Bicarbonate loading	2.6	1.0[c]	34.5	39.5	121.70	112.30	7.76	7.74	16.5	13.5	4.8	14.1[c]
	±0.2	±0.3	±2.8	±4.5	±10.34	±8.36	±0.3	±0.2	±3.3	±2.8	±1.0	±0.3

[a] Values are the means ± SEM (N = 6 control animals, 8 ARF animals). Abbreviation not defined in Table 1 is: V/GFR, fractional water excretion.
[b] $P < 0.02$, compared with controls.
[c] $P < 0.01$, compared with controls.

Table 5. Urine-blood P_{CO_2} in acute renal failure during neutral phosphate infusion[a]

	GFR *ml/min*		U-B P_{CO_2} *mmHg*		P_{PO_4} *mmoles/liter*		U_{PO_4} *mmoles/liter*		Urine pH		FE_{PO_4} %		FE_{Na} %	
Experimental period	C	ARF	C	ARF	C	ARF	C	ARF	C	ARF	C	ARF	C	ARF
Control	2.2	0.6[c]	− 8.9	−10.5	2.5	2.7	21.3	38.5[b]	6.07	5.32[b]	8.9	63.9[c]	1.01	0.8
	±0.2	±0.2	± 6.9	± 1.7	±0.1	±0.3	± 8.0	±10.2	±0.29	±0.07	± 2.5	±11.7	±0.2	± 0.3
P	<0.02	NS	< 0.001	< 0.01	<0.001	<0.05	< 0.01	< 0.05	<0.02	<0.001	< 0.001	NS	<0.001	< 0.05
	2.8	0.8	87.4	44.8[b]	5.0	4.9	74.3	52.4[b]	7.08	7.04	68.8	75.4	9.4	10.6
Phosphate infusion	±0.1	±0.2	±16.9	± 7.6	±0.3	±0.7	± 8.7	±10.6	±0.02	±0.06	± 3.6	± 6.8	±0.7	± 3.9

[a] Values are the means ± SEM (N = 6 control animals, 6 ARF animals).
[b] $P < 0.05$, compared with controls.
[c] $P < 0.001$, compared with controls.

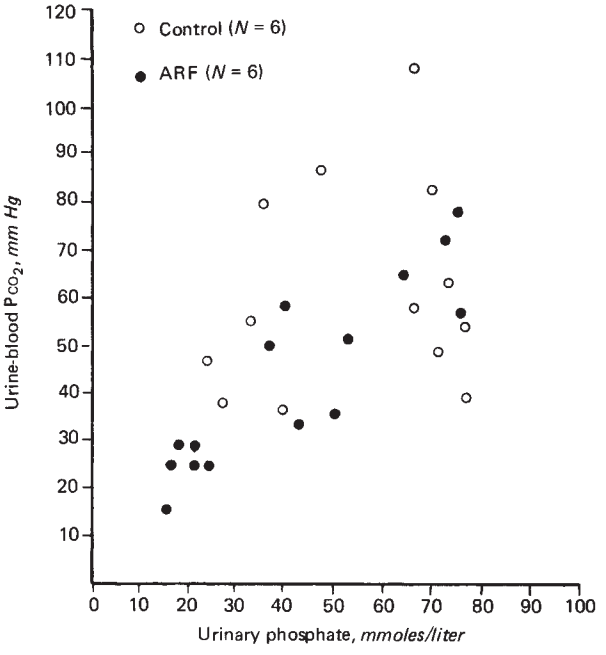

Fig. 4. *Plot of U-B PCO_2 against urinary phosphate concentration.* For symbol identification, see legend to Fig. 1.

animals ($y = 1.09x + 7.7, r = 0.83, P < 0.01$) did not differ from that obtained in controls ($y = 0.77x + 10.8, r = 0.89, P < 0.01$) both as regards slope and intercept. The higher U-B P_{CO_2} value observed in control animals resulted, therefore, clearly from the higher final U_{PO_4} concentration achieved in these animals. These data also suggest that distal acidification remains intact in this form of ARF. This notion is further supported by the fact that spontaneous urinary pH in these ARF animals was less than 5.5.

Net acid excretion. Table 6 contains the data from these experiments. The GFR was markedly depressed in rats with ARF. Blood pH following ammonium chloride ingestion was below 7.25, and simultaneous urinary pH was less than 5.5 both in ARF and control animals. Blood P_{CO_2} and bicarbonate concentration was similar in experimental and control animals. The net acid excretion and ammonium excretion per milliliter GFR was similar in control and ARF animals. Titratable acid excretion per milliliter GFR was significantly higher in animals with ARF. These data corroborate that distal acidification in animals with glycerol-induced ARF is intact (see U-B P_{CO_2} data above).

Potassium excretion. Table 7 summarizes the potassium data from six experimental groups. Note that plasma potassium was not elevated in any of the experimental groups. Fractional potassium excretion in animals with ARF was greater than 100% in the studies in hydropenia, and in the glucose, 3% saline, and H_2O infusion studies. Urine flow (V) and $U_{Na}V$ values were similar in all groups except when phosphate was infused.

Free water excretion (C_{H_2O}). The data from these experiments are summarized in Table 8. The animals with ARF showed a marked decline in GFR when compared with controls. The minimal urinary osmolality (U_{min}) achieved during maximal water diuresis was significantly higher in ARF animals. Urine flow and osmolar clearance were similar in

Table 6. Net acid excretion in acute renal failure[a]

Experimental animals	GFR *ml/min*	TA/GFR *μEq/ml*	NH_4^+/GFR *μEq/ml*	Net acid/GFR *μEq/ml*	Blood pH	Blood P_{CO_2} *mm Hg*	Blood HCO_3 *mEq/liter*	Urine pH
Controls (N = 7)	1.2 ± 0.1	0.7 ± 0.2	1.3 ± 0.4	2.0 ± 0.5	7.23 ± 0.03	33.9 ± 0.9	13.9 ± 1.2	5.43 ± 0.04
P	< 0.01	< 0.01	NS	NS	NS	NS	NS	NS
ARF (N = 5)	0.3 ± 0.1	1.4 ± 0.2	1.1 ± 0.3	2.4 ± 0.4	7.21 ± 0.05	33.6 ± 1.2	13.6 ± 1.9	5.33 ± 0.01

[a] Values are the means ± SEM. Abbreviations not defined in Table 1 are: TA/GFR, titratable acid excretion per milliliter GFR; NH_4^+/GFR, ammonium excretion per milliliter GFR; Net acid/GFR, net acid excretion per milliliter GFR.

Table 7. Potassium excretion in acute renal failure[a]

Experimental periods	GFR *ml/min*		P_K *mEq/liter*		FE_K %		U_KV *μEq/min*		FE_{Na} %		$U_{Na}V$ *μEq/min*		V *ml/min*	
	C	ARF	C	ARF	C	ARF	C	ARF	C	ARF	C	ARF	C	ARF
Hydropenic	2.9	0.5[e]	2.9	2.7	27.9	164.0[e]	1.8	1.5	0.6	6.5[b]	2.0	1.4	0.03	0.03
(*N* = 10,16)	±0.9	±0.1	±0.2	±0.2	± 5.4	± 13.5	±0.2	±0.2	±0.1	± 2.4	± 0.3	± 0.3	±0.01	±0.01
Bicarbonate infusion	2.6	1.0[d]	3.8	3.2	46.9	91.0[e]	4.5	3.3	9.1	16.1[b]	33.3	26.2	0.14	0.13
(*N* = 6,8)	±0.2	±0.3	±0.1	±0.2	± 2.1	± 12.7	±0.6	±0.5	±1.5	± 3.1	± 4.3	± 3.7	±0.02	±0.02
Glucose infusion	1.9	0.4[e]	3.4	3.3	32.2	104.0[e]	1.6	1.0	0.7	6.3[b]	2.8	2.0	0.05	0.04
(*N* = 8,11)	±0.3	±0.1	±0.3	±0.1	± 7.9	± 12.0	±0.3	±0.1	±0.4	± 2.4	± 1.4	± 0.7	±0.01	±0.01
Phosphate infusion	2.8	0.8[e]	3.1	3.9[e]	42.0	95.4	3.6	2.2[d]	9.4	10.6	40.3	7.3[e]	0.14	0.05[e]
(*N* = 6,6)	±0.1	±0.2	±0.1	±0.2	± 2.7	± 24.7	±0.2	±0.4	±0.7	± 3.9	± 2.5	± 1.0	±0.02	±0.01
H_2O diuresis	2.9	0.7[e]	3.2	3.1	33.2	127.7[d]	2.9	2.5	2.3	14.0[d]	9.0	10.8	0.33	0.25
(*N* = 6,8)	±0.5	±0.1	±0.1	±0.1	± 8.1	± 22.6	±0.6	±0.3	±0.4	± 2.6	± 1.8	± 0.8	±0.05	±0.02
3% Sodium chloride infusion	2.3	0.5[e]	2.4	3.3	53.5	143.4[e]	2.1	1.5	8.1	29.1[d]	38.8	24.0	0.12	0.10
(*N* = 6,9)	±0.3	±0.1	±0.3	±0.4	±16.7	± 24.1	±0.3	±0.5	±1.3	± 5.3	± 4.0	± 7.6	±0.03	±0.03

[a] Values are the means ± SEM. Abbreviations are defined in Table 1. *N* denotes number of control and ARF animals, respectively.
[b] $P < 0.05$, compared with controls.
[c] $P < 0.02$, compared with controls.
[d] $P < 0.01$, compared with controls.
[e] $P < 0.001$, compared with controls.

Table 8. Free water excretion (C_{H_2O}) in acute renal failure[a]

Experimental animals	GFR *ml/min*	V *ml/min*	U_{min} *mosm/kg H_2O*	C_{osm} *ml/min*	C_{H_2O} *ml/min*	C_{H_2O}/GFR %	$(C_{H_2O} + C_{Na})$/GFR %	V/GFR %
Controls (*N* = 6)	2.9 ± 0.5	0.33 ± 0.05	76.7 ± 4.3	0.12 ± 0.02	0.22 ± 0.03	7.5 ± 0.4	9.9 ± 0.7	11.7 ± 0.9
P	< 0.001	NS	< 0.01	NS	< 0.05	< 0.001	< 0.001	< 0.001
ARF (*N* = 8)	0.7 ± 0.1	0.25 ± 0.02	142.1 ± 11.2	0.13 ± 0.01	0.13 ± 0.02	17.8 ± 1.2	30.7 ± 2.5	38.5 ± 3.4

[a] Values are the means ± SEM. Abbreviations not defined in Table 1 are: U_{min}, minimal urinary osmolality; $C_{H_2O} + C_{Na})$/GFR, fractional distal sodium delivery; V/GFR = fractional water excretion.

Table 9. Free water reabsorption ($T^c_{H_2O}$) in acute renal failure[a]

Experimental animals	GFR *ml/min*	V *ml/min*	U_{max} *mOsm/kg H_2O*	C_{Osm} *ml/min*	$T^c_{H_2O}$ *ml/min*	$T^c_{H_2O}$/GFR %	C_{Osm}/GFR %	V/GFR %
Controls (*N* = 6)	2.3 ± 0.3	0.124 ± 0.031	2214 ± 155	0.25 ± 0.04	0.13 ± 0.02	6.9 ± 0.3	11.4 ± 1.9	6.2 ± 1.4
P	< 0.001	NS	< 0.001	< 0.05	< 0.001	NS	<0.02	<0.02
ARF (*N* = 10)	0.5 ± 0.1	0.096 ± 0.028	586 ± 60	0.12 ± 0.04	0.03 ± 0.01	6.9 ± 1.9	36.2 ± 6.7	27.1 ± 6.3

[a] Values are the means ± SEM. Abbreviations not defined in Table 1 are: U_{max} = maximal urinary osmolality; V/GFR = fractional water excretion.

control and ARF animals. Fractional free water clearance (C_{H_2O}/GFR), fractional distal sodium delivery ($C_{H_2O} + C_{Na}$/GFR) and fractional water excretion (V/GFR) were significantly higher in ARF rats. When fractional free water excretion was examined as a function of fractional distal sodium delivery, it became apparent that the experimental points did not overlap. Therefore, a valid statement regarding differences in overall diluting capacity at any one level of distal delivery cannot be made.

Free water reabsorption ($T^c_{H_2O}$). Table 9 summarizes the data from this experiment. The GFR was significantly decreased in ARF animals. The maximal urinary osmolality (U_{max}) achieved in ARF animals (after water deprivation and vasopressin administration) was strikingly depressed when compared with controls. Urine flow and fractional free water reabsorption ($T^c_{H_2O}$/GFR), however, was similar in ARF and control rats. When fractional free water reabsorption was examined as a function of fractional osmolar clearance (C_{Osm}/GFR), no overlap in experimental points was obtained. Therefore, overall concentrating capacity of ARF animals at any one level of distal delivery could not be compared with that of controls.

Sodium reabsorption and Na-K-ATPase activity. Table 10 and Fig. 5 show the data from these experiments. Both the animals with ARF and those with constriction of the suprarenal abdominal aorta (coarctation) showed, 24 hours after the respective procedure, a marked depression in GFR and a rise in BUN when compared with controls. This decline

Table 10. Effect of acute renal failure and coarctation of the aorta on renal function[a]

Experimental animals	GFR *ml/min*	BUN *mg/dl*	FE_{Na} %	FE_K %	V/GFR %	Wt *g*
Controls (N = 13)	2.8 ± 0.3	16.7 ± 1.0	0.6 ± 0.1	23.0 ± 3.2	1.1 ± 0.7	339 ± 25
P	< 0.001	< 0.001	NS	< 0.001	< 0.01	NS
ARF (N = 21)	0.5 ± 0.1	50.0 ± 5.5	4.7 ± 1.7	169.5 ± 13.1	9.9 ± 1.7	300 ± 14
P	< 0.01	NS	NS	< 0.001	< 0.05	NS
Coarctation (N = 7)	0.9 ± 0.1	31.2 ± 4.2	0.9 ± 0.6	37.7 ± 10.7	1.9 ± 0.9	312 ± 12
P (vs controls)	< 0.01	< 0.001	NS	NS	NS	NS

[a] Values are the means ± SEM. V/GFR is fractional water excretion. For enzyme data see Fig. 7.

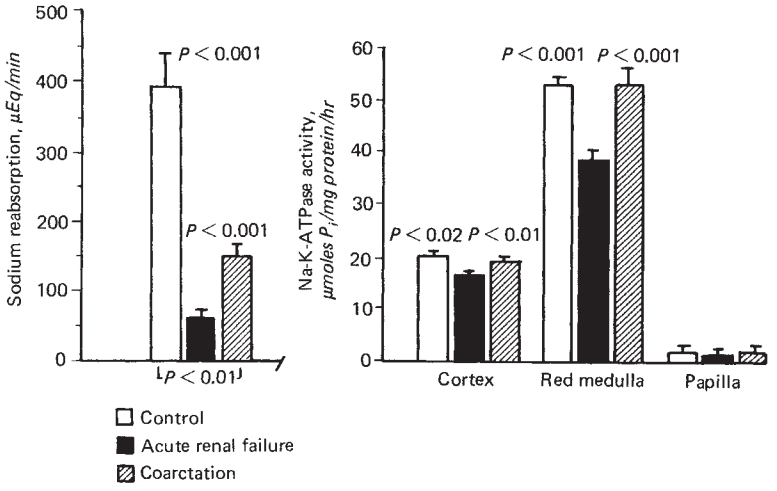

Fig. 5. Left panel *Absolute tubular sodium reabsorption (T_{Na}) in control* (open bars, N = 13), *ARF* (black bars, N = 21), *and coarctation animals* (crosshatched bars, N = 7). **Right panel** *Na-K-ATPase specific activities in renal cortex, red medulla, and papilla in the same experimental animals.*

in GFR was associated with a parallel depression in absolute tubular sodium reabsorption (T_{Na}; see Fig. 5). Note that the Na-K-ATPase specific activity in cortex and red medulla from ARF animals was markedly depressed. A 24-hour reduction of T_{Na} subsequent to aortic clipping was not associated with a decline in enzyme activities. The decline in Na-K-ATPase specific activity resulted from a loss in active enzyme per milligram of protein and not from alterations in enzyme kinetics, that is, apparent Km's for ATP, sodium, or potassium (see Methods section). These data indicate that this type of ARF is associated with a direct loss in Na-K-ATPase specific activity (in cortex and red medulla) and that this decline in activity does not result (at least not exclusively) from a primary depression in T_{Na}.

Histology. Figure 6 shows light- and electron-microscopic photographs taken from control (panels A and C) and ARF (panels B and D) animals 24 hours after glycerol or saline injection. Note that kidneys from rats with ARF showed a diffuse damage of proximal tubules, whereas glomeruli and many distal tubules were intact and comparable with controls (panels A and B). Panel D shows a proximal tubule from an animal with ARF. The cellular damage is characterized by a complete loss of the brush-border, marked mitochondrial swelling, and cytoplasmic vacuolization. Panel C shows a normal proximal tubular segment.

Discussion

The objective of the present study was to systematically examine tubular function in glycerol-induced ARF. Most previous investigations of ARF have concentrated on the study of the pathogenesis and pathophysiology of filtration failure, leaving the functional expression of the syndrome incompletely elucidated [1, 2]. The glycerol model of this syndrome was chosen because it has been extensively studied and is felt to closely resemble the human form of pigment-induced ARF [2].

In the so called "nephrotoxic" form of human or experimental ARF, various defects of tubular func-

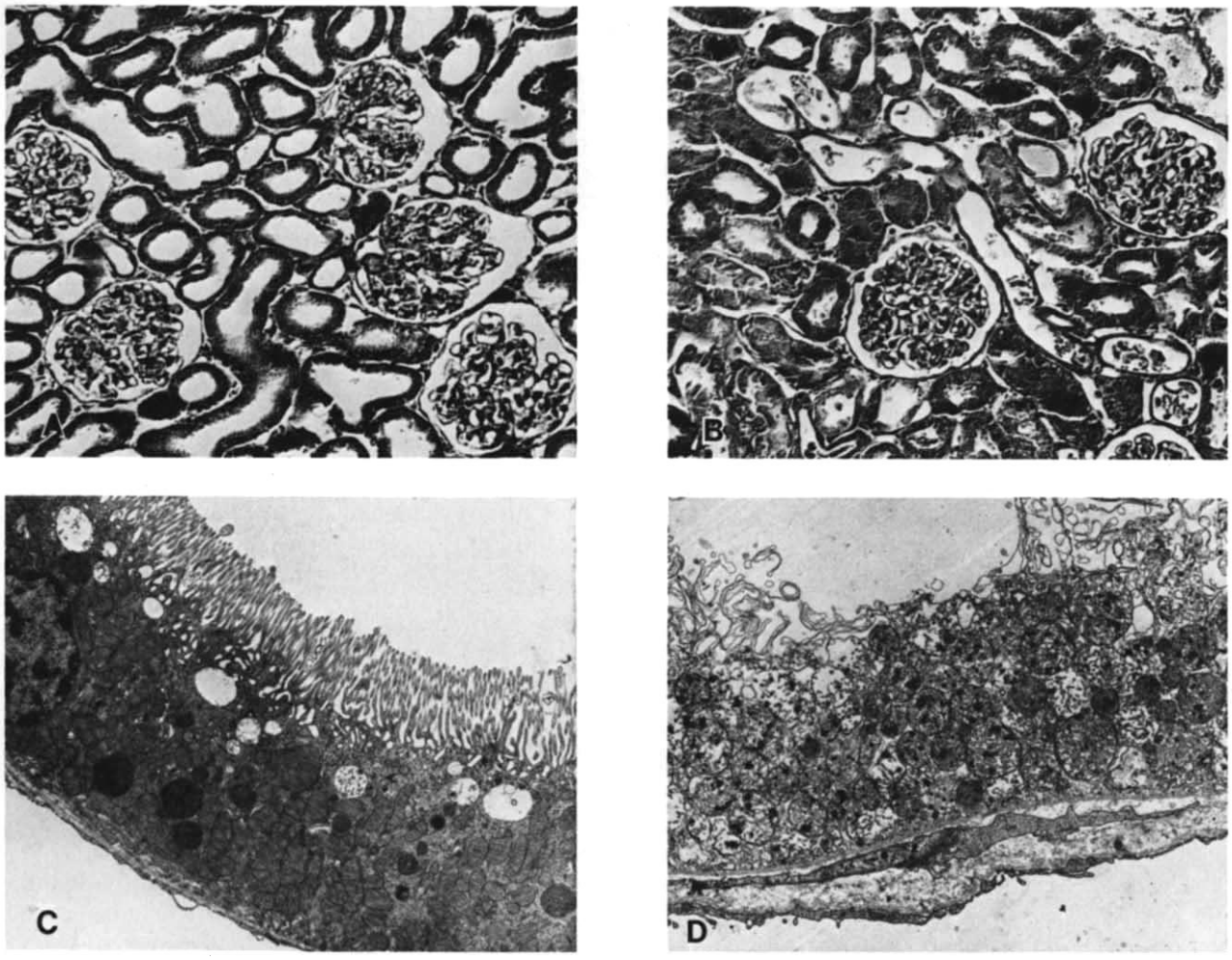

Fig. 6. *Photomicrographs of histologic sections from rat kidney cortex.* **A** shows control. **B** shows ARF animals (Trichrome stain; original magnification, ×80). **C** shows electronmicrograph of proximal tubule from control. **D** represents ARF animals (original magnification of panel C is ×5,700 and D is ×8,900).

tion were reported. Most frequent among these defects were glycosuria, aminoaciduria, proteinuria, decreased PAH extraction, and defective concentrating ability [2, 14–24]. No detailed quantitative study, however, of these tubular function abnormalities is presently available. We also attempted to correlate the changes in tubular function with the histologic pattern of renal cell damage and alterations in Na-K-ATPase activity in the glycerol model of ARF. A similar approach in other models of ARF has been taken by Baines [18] and others [19, 25].

The GFR 24 hours after glycerol fell by 60 to 90% as measured by the clearance of iothalamate. The clearance of iothalamate did not differ from that of inulin (data not shown). Many investigators in the past assessed renal function in this model of ARF only by measurement of BUN concentrations or by determination of single nephron GFR by micropuncture technique. Our data and those of others using different clearance markers [2, 26] agree in the magnitude of glomerular filtration loss seen in nondehydrated rats given glycerol.

Our data suggest that the animals with ARF at the time of study were not volume expanded but rather volume contracted or normovolemic. This observation is important in interpreting the clearance data because proximal tubular reabsorption of bicarbonate and glucose falls markedly with effective arterial blood volume expansion [5, 27]. This conclusion as to the state of volume was reached from the following evidence. The urine flow in animals with ARF was generally not different from that of controls (see Table 7). None of the experimental animals developed oliguria. The rats with ARF experienced a mean weight loss of approximately 12.5 g

(range, 10 to 20 g) within the 24 hours of glycerol administration, whereas the controls lost a mean of 6.5 g (range, 3 to 8 g) during the same observation period. The food and water intake by the ARF animals was very low compared with controls. The mean arterial blood pressure at the time of study was not different in experimental and control rats (data not shown). The hematocrit 24 hours after glycerol or saline injection was not lower in rats with ARF (42 ± 3 vs. 44 ± 2%, NS). A fall in hematocrit due to hemolysis was probably obscured by concomitant hemoconcentration. All these facts rule out volume expansion at the time of study.

Therefore, the marked depression in glucose, bicarbonate, and phosphate reabsorption observed in animals with glycerol-induced ARF provides clear evidence for a diffuse defect in proximal tubular function, which closely resembles that observed in nephrotoxic forms of ARF [2, 14–24, 28]. This proximal tubular defect also affects the organic acid transport system, for both PAH secretion and PAH extraction were strikingly decreased. Abnormalities in active PAH transport have also been demonstrated in in vitro studies that use renal cortical slices from rats with glycerol-induced ARF [29]. It is of interest that the bicarbonate reabsorption at normal concentrations of blood bicarbonate was intact and that only the "tubular maximum" for bicarbonate was reduced. This might have been in part due to a less severe impairment in renal function observed in this group of animals. Indeed, the rats with a more severe degree of ARF showed a depression in tubular bicarbonate reabsorption at normal blood bicarbonate levels. In contrast, both glucose and PAH transport were markedly depressed in animals with only mild ARF. This suggests that the integrity of proximal tubular transport in this syndrome can be most sensitively assessed by examining the renal handling of either glucose or PAH (that is, when clearance techniques are used).

These data showing marked depression in proximal tubular function are supported by examination of the renal histology, which demonstrated that the main site of diffuse tubular cell damage was the proximal convolution. The pars recta showed less severe subcellular and cellular alterations in a more patchy distribution. Cells of the thick ascending limb showed only minor changes, including mitochondrial swelling and cytoplasmic vacuolization. Tubular cell (presumably proximal) debris, and pigment casts were found in the pars recta, the loop of Henle, and the collecting tubules. The epithelium of the latter appeared, however, entirely intact. Cast formation and cell swelling (with occlusion of the proximal tubular lumen) was frequently noted and probably produced some degree of tubular obstruction. Similar findings have been previously reported by others [30–32]. Whether this mechanism (that is, obstruction) plays a significant role in the reduction of GFR in this model of ARF is controversial [2]. It is noteworthy that the damages observed in the proximal tubules of superficial nephrons were similar to those observed in proximal tubules of deep nephrons.

The defects of proximal tubular function correlated well with both the site of tubular cell injury (that is, the proximal tubule, see above) and a loss in Na-K-ATPase specific activity of the renal cortex. This decline in specific activity was not due to alterations in enzyme kinetics and was paralleled by a fall in the specific activity of magnesium-ATPase (80% of control), succinic dehydrogenase (76% of control; mitochondrial marker enzyme), and 5′-nucleotidase (78% of control; microsomal marker) (data not given)[2]. Similar depressions in enzyme activity have been reported by Baines [18] and Schmidt and Dubach [25], who studied heavy metal- and folate-induced ARF, respectively. This nonspecificity of loss in enzyme activities seems to indicate that these changes are the result of extensive tubular cell injury.

To examine whether the decline in absolute tubular sodium reabsorption (T_{Na}) in ARF animals could have caused the fall in Na-K-ATPase activity, we reduced the T_{Na} in normal rats by constricting the abdominal aorta (Table 10, Fig. 5). This experiment was necessary because we demonstrated previously that a prolonged decrease in T_{Na} (that is, lasting for several days) resulted in a parallel fall in Na-K-ATPase activity [4]. Because a reduction in T_{Na} of 24 hr's duration (produced by aortic constriction) had no effect on renal Na-K-ATPase activity (Fig. 5), it appears unlikely that the loss in enzyme activity seen in ARF resulted from a primary fall in T_{Na}. This observation is in agreement with the data of Fisher et al [35].

Distal acidification was assessed in several ways. All animals with ARF were able to spontaneously reduce their urinary pH to less than 5.5. Following several days of ammonium chloride administration,

[2] Magnesium-ATPase activity was measured as part of the Na-K-ATPase assay. The activity of the succinic dehydrogenase was determined in whole homogenates according to Kun and Abood [33] and that of the 5′-nucleotidase according to Dixon and Purdom [34] by using light microsomes [4].

both control and ARF animals were equally acidotic, and their titratable acidity and ammonium chloride excretion per milliliter of GFR were similar. These data suggest that distal acidification was unimpaired.

Maximal urinary alkalinization with bicarbonate (Tables 2 and 4) demonstrated clearly that animals with ARF can achieve a U-B P_{CO_2} gradient comparable to that of controls. Similarly, during the infusion of neutral phosphate, U-B P_{CO_2} rose linearly and identically with the urinary phosphate concentration both in experimental and control animals. These data taken as a whole clearly demonstrate, therefore, that animals with glycerol-induced ARF have a preserved capacity for distal acidification.

The excretion of potassium under various experimental conditions was intact (see Table 7). Note that none of the animals with ARF was hyperkalemic at the time of study. When examined under hydropenic conditions or when measured during bicarbonate, glucose, water, or 3% sodium administration, absolute potassium and sodium excretion were similar in ARF and control animals. In addition, urine flows under the same experimental conditions were not different. It could be argued that a defective potassium reabsorption in more proximal nephron segments (that is, proximal to the distal secretory site) could amount for the relatively normal capacity to eliminate potassium. A fractional potassium excretion of greater than 100% indicates, however, that the potassium secretory mechanism is intact. Histologic examination of the collecting tubules showed no evidence of tubular cell damage both on light and electron microscopy. This likely explains the intact acidifying and potassium-secreting capacity observed in ARF animals.

Animals with ARF showed a marked depression both in maximal urinary concentrating and diluting capacity. This is in agreement with earlier studies by Bowman and Foulkes [21] using the uranyl nitrate model of ARF.

Summary. The present study demonstrates that glycerol-induced, nonoliguric ARF is characterized by a diffuse defect in proximal tubular function, histologic damage most pronounced at this site of the nephron, and by a depression in the activity of Na-K-ATPase and other enzymes found in high concentrations in the proximal nephron. It is evident, therefore, that the depression of proximal tubular function seen in the glycerol model of ARF is indistinguishable from that seen in nephrotoxic forms of this syndrome. Distal tubular function as assessed by measurement of acidification and potassium secretion remains intact, whereas both urinary diluting and concentrating ability are defective.

Acknowledgments

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